Neurotoxicity of Flatweed: the presumptive cause of equine stringhalt

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In 2009, aerial parts of *Hypochoeris radicata* (Figure 1) were harvested from sites associated with cases of stringhalt (Figure 2) during the previous summer and autumn. Two hundred gram amounts of plants were incubated either with the stressor, 3mM $CuCl_2$ or with water for 24 hours resulting in 'stressed' exudates and 'unstressed' exudates. The exudates were collected and extracted into ethyl acetate, dried, weighed and reconstituted in 0.1% dimethyl sulfoxide/culture media and filter-sterilized.

Figure 1: *Hypochaeris radicata, also known as the flatweed, 'catsear', is a member of the Asteraceae plant family. (Photographs courtesy of Dr. Kongara)*

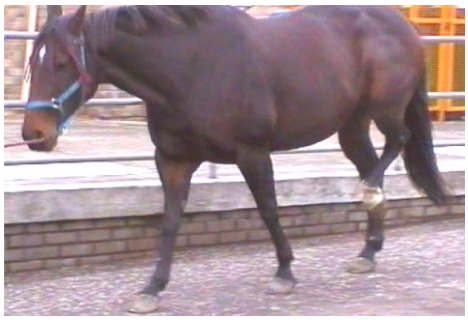

Figure 2: *Horse showing classical signs of stringhalt with exaggerated flexion of the hind limb. (Photograph courtesy of Prof. J. Mayhew)*

Simultaneously, three different types of cell cultures were established: 1) mixed neuronal and glial cultures from mouse spinal cord, representing the central nervous system; 2) mixed neuronal and glial cultures from mouse spinal ganglia, representing the peripheral nervous system; 3) mouse skin fibroblasts representing non-neuronal cell types. The extracts from stressed and unstressed *H. radicata* leaves, at various concentrations, were added to the three different cultures. Negative controls included media only and vehicle only. The neurotoxin, repin, was used as a positive control. Repin is derived from Russian Knapweed (*Centaurea repens)*, a plant in the same family (*Asteraceae*) as *H. radicata*; it causes a neurological disease of horses in North America.

The nerve cells and supporting cells in these cultures were evaluated for evidence of cell death by light microscopy, and biochemically using the Lactate Dehydrogenase (LDH) assay. The experiments were repeated in triplicate, using three separate batches of *H. radicata*. We found that *H. radicata* extracts caused dose-related toxicity in all cell cultures, with the stressed extract being more potent than the non-stressed extract. Similarly, repin caused dose -related toxicity in all three types of cultured cells. We collected plant samples from the same farms in 2010. aiming to ascertain how repeatable the *in vitro* toxicity studies were. We found the same toxicity in the 2010 plants, strengthening our data.

Our findings support the hypothesis that *H. radicata* produces substances that are toxic to mammalian nerve cells, especially when the plants are stressed. Based on the outcome of some final tests to ensure that the toxic effect of the plant extract is specific, we will then try to identity the putative neurotoxin.